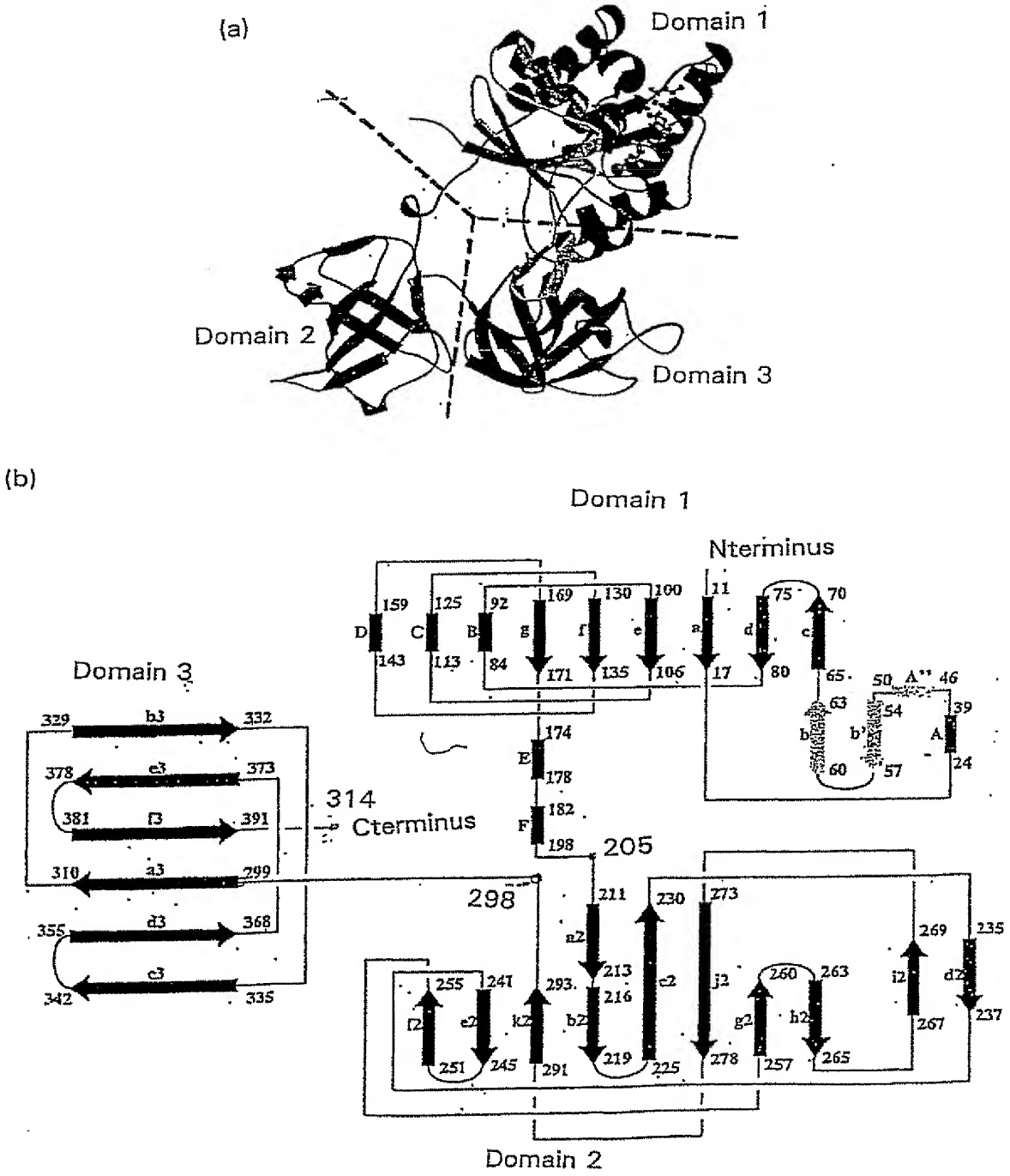
Figur 1


(a)
Domain 1
Domain 2
Domain 3
(b)
Domain 1
Nterminus
Domain 3
314
Cterminus
205
298
Domain 2

Figur 2

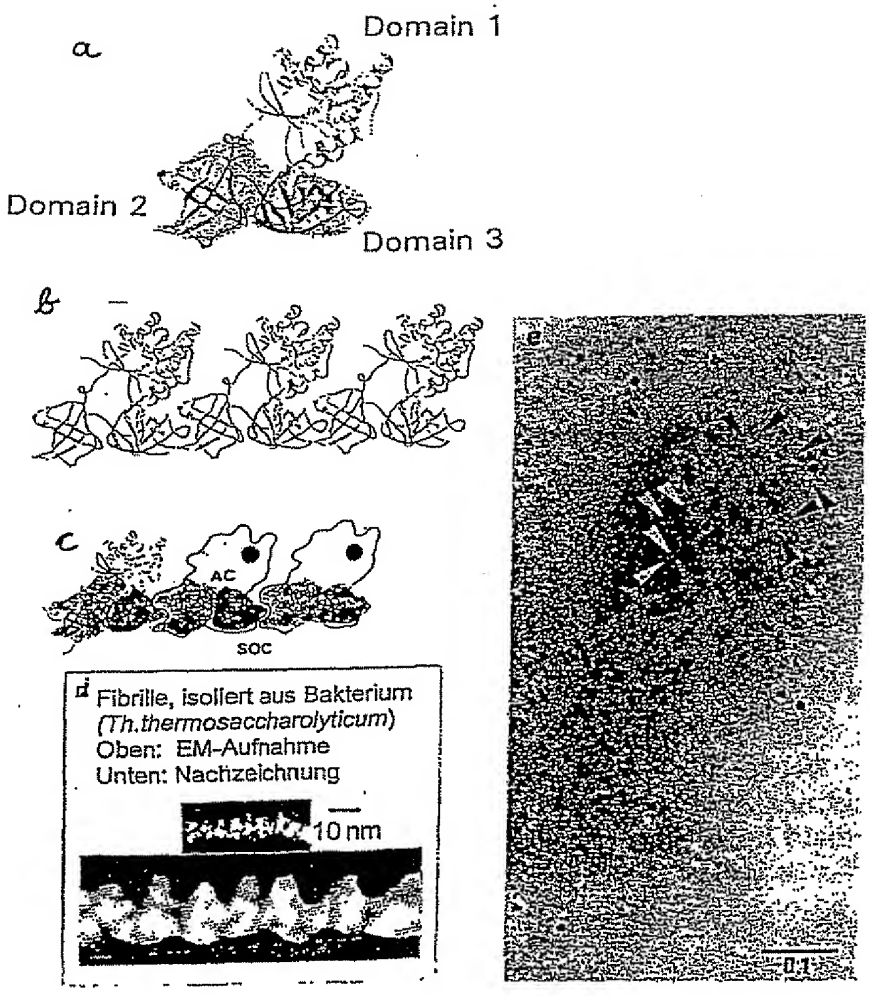

a
Domain 1
Domain 2
Domain 3
b
c
AC
SOC
d
Fibrille, isoliert aus Bakterium
(Th.thermosaccharolyticum)
Oben: EM-Aufnahme
Unten: Nachzeichnung
10 nm
e
0.1

Fig. 3

(a1)
Vektor pEGFP (Clontech):

```
          BsrGI        STOP                          EcoRI
GAC GAG CTG TAC AAG TAA AGC GGC CGC GAC TCT AGA ATT CCA
CTG CTC GAC ATG TTC ATT TCG CCG GCG CTG AGA TCT TAA GGT
```

BsrGI-Schnittstelle:

```
  T    GTACA
  ACATG    T
```

EcoRI-Schnittstelle:

```
    G    AATTC
    CTTAA    G
```

Synthetisch hergestelltes Oligonukleotid zur Einklonierung des His-Tags in den Vektor:

```
5'BsrGI                                          BsrGI        EcoRI 3'
G TAC AAG CTT CAT CAC CAT CAC CAT CAC TAA CTG TAC AAG TAAG
      TTC GAA GTA GTG GTA GTG GTA GTG ATT GAC ATG TTC ATTCTTAA
3'                                                              5'
  Tyr-Lys-Leu-His-His-His-His-His-His-STOP-
```

Ergebnis: pEGFP(His)

(a2)

Vektor pEGFP(His):

```
GCC TGC AGG -%- ACC ATG GTG
CGG ACG TCC -%- TGG TAC CAC
```

PstI-Schnittstelle:

```
CTGCA     G
G     ACGTC
```

NcoI-Schnittstelle:

```
C     CATGG
GGTAC     C
```

Fusionsstellen zum EF-Tu-Gen:

```
                  Start EF-Tu                                  Start EGFP
5'    PstI                                        HindIII    NcoI        3'
ACT AGC TGC AGC ATG TCT AAA -%- CTG GGC AAG CTT ACC ATG GTG
TGA TCG ACG TCG TAC AGA TTT -%- GAC CCG TTC GAA TGG TAC CAC
3'                                                            5'
Thr-Ser-Cys-Ser-Met-Ser-Lys-----Leu-Gly-Lys-Leu- Thr-Met-Val
```

(a3)

Fusionsstellen zur Domäne 3:

```
                                                  Cys  HindIII     NcoI    3'
5'     PstI                                                                
ACT AGC TGC AGC GCT AAG CCG -%- CTG GGC TGC AAG CTT ACC ATG GTG
TGA TCG ACG TCG CGA TTC GGC -%- GAC CCG ACG TTC GAA TGG TAC CAC
                                                                        5'
3''
Thr-Ser-Cys-Ser-Ala-Lys-Pro-----Leu-Gly-Cys-Lys-Leu-Thr-Met-Val
```

Sequenz des Konstrukts EF-Tu-GFP-His im Vektor pEGFP (Clontech) (SEQ ID NO:1)

(b1)

pEGFP-Vektor:
AGCGCCCAAT ACGCAAACCG CCTCTCCCCG CGCGTTGGCC GATTCATTAA TGCAGCTGGC ACGACAGGTT TCCCGACTGG
AAAGCGGGCA [redacted] T CACAC[redacted]A ACAGCTATGA CCATGATTAC GCCAAGCTTG
CATGC[redacted]C

EF-Tu:
ATGTCTAAAG AAAAATTTGA ACGTACAAAA CCGCACGTTA ACGTTGGTAC TATCGGCCAC GTTGACCACG GTAAAACTAC
TCTGACCGCT GCAATCACCA CCGTACTGGC TAAAACCTAC GGCGGTGCTG CTCGTGCATT CGACCAGATC GATAACGCGC
CGGAAGAAAA AGCTCGTGGT ATCACCATCA ACACTTCTCA CGTTGAATA[redacted] GACACCCCGA CCCGTCACTA CGCACACGTA
GACTGCCCGG GGCACGCCGA CTATGTTAAA AACATGATCA CCGGTGCTGC TCAGATGGAC GGCGCGATCC TGGTAGTTGC
TGCGACTGAC GGCCCGATGC CGCAGACTCG TGAGCACATC CTGCTGGGTC GTCAGGTAGG CGTTCCGTAC ATCATCGTGT
TCCTGAACAA ATGCGACATG GTTGATGACG AAGAGCTGCT GGAACTGGTT GAAATGGAAG TTCGTGAACT TCTGTCTCAG
TACGACTTCC CGGGCGACGA CACTCCGATC GTTCGTGGTT CTGCTCTGAA AGCGCTGGAA GGCGACGCAG AGTGGGAAGC
GAAAATCCTG GAACTGGCTG GCTTCCTGGA TTCTTA[redacted]ATT CCGGAACCAG AGCGTGC[redacted]AT TGACAAGCCG TTCCTGCTGC
CGATCGAAGA CGTATTCTCC ATCTCCGGTC GTGGTACCGT TGTTACCGGT CGTGTAGAAC GCGGTATCAT CAAAGTTGGT
GAAGAAGTTG AAATCGTTGG TATCAAAGAG ACTCAGAAGT CTACCTGTAC TGGCGTTGAA ATGTTCCGCA AACTGCTGGA
CGAAGGCCGT GCTGGTGAGA ACGTAGGTGT TCTGCTGCGT GGTATCAAAC GTGAAGAAAT CGAACGTGGT CAGGTACTGG
CTAAGCCGGG CACCATCAAG CCGCACACCA AGTTCGAATC TGAAGTGTAC ATTCTGTCCA AAGATGAAGG CGGCCGTCAT
ACTCCGTTCT TCAAAGGCTA CCGTCCGCAG TTCTACTTCC GTACTACTGA CGTGACTGGT ACCAT[redacted]GAAC TGCCGGAAGG
CGTAGAGATG GTAATGCCGG GCGACAACAT CAAAATGGTT GTTACCCTGA TCCACCCGAT CGCGATGGAC GACGGTCTGC
GTTTCGCAAT CCGTGAAGGC GGCCGTACCG TTGGCGCGGG CGTTGTAGCT AAAGTTCTGG GC

pEGFP-Vektor:
AAGCTTA[redacted]G

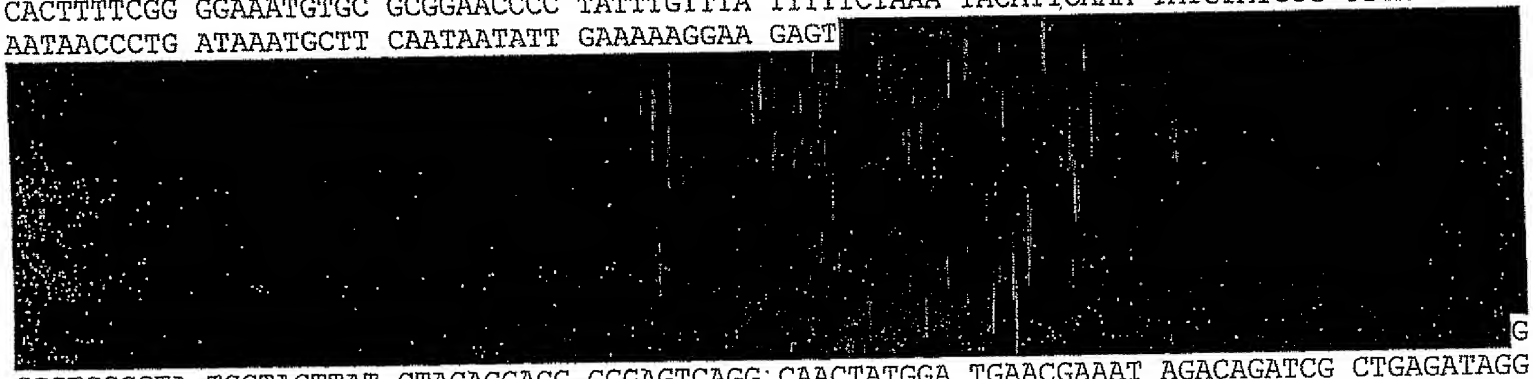
GFP:
[redacted]TGAGCA AGGGCGAGGA GCTGTTCACC GGGGTGGTGC CCATCCTGGT CGAGCTGGAC GGCGACGTAA ACGGCCACAA

REPLACEMENT SHEET

GTTCAGCGTG TCCGGCGAGG GCGAGGGCGA TGCCACCTAC GGCAAGCTGA CCCTGAAGTT CATCTGCACC ACCGGCAAGC
TGCCCGTGCC CTGGCCCACC CTCGTGACCA CCCTGACCTA CGGCGTGCAG TGCTTCAGCC GCTACCCCGA CCACATGAAG
CAGCACGACT TCTTCAAGTC CGCCATGCCC GAAGGCTACG TCCAGGAGCG CACCATCTTC TTCAAGGACG ACGGCAACTA
CAAGACCCGC GCCGAGGTGA AGTTCGAGGG CGACACCCTG GTGAACCGCA TCGAGCTGAA GGGCATCGAC TTCAAGGAGG
ACGGCAACAT CCTGGGGCAC AAGCTGGAGT ACAACTACAA CAGCCACAAC GTCTATATCA TGGCCGACAA GCAGAAGAAC
GGCATCAAGG TGAACTTCAA GATCCGCCAC AACATCGAGG ACGGCAGCGT GCAGCTCGCC GACCACTACC AGCAGAACAC
CCCCATCGGC GACGGCCCCG TGCTGCTGCC CGACAACCAC TACCTGAGCA CCCAGTCCGC CCTGAGCAAA GACCCCAACG
AGAAGCGCGA TCACATGGTC CTGCTGGAGT TCGTGACCGC CGCCGGGATC ACTCTCGGCA TGGACGAGCT GTACAAG

His-Tag:
CTTCATCACC ATCACCATCA CTAACTGTAC AAGTAAGAATTC

pEGFP-Vektor:
CAACTGAGCG CCGGTCGCTA CCATTACCAA CTTGTCTGGT GTCAAAAATA ATAGGCCTAC TAGTCGGCCG TACGGGCCCT
TTCGTCTCGC GCGTTTCGGT GATGACGGTG AAAACCTCTG ACACATGCAG CTCCCGGAGA CGGTCACAGC TTGTCTGTAA
GCGGATGCCG GGAGCAGACA AGCCCGTCAG GGCGCGTCAG CGGGTGTTGG CGGGTGTCGG GGCTGGCTTA ACTATGCGGC
ATCAGAGCAG ATTGTACTGA GAGTGCACCA TATGCGGTGT GAAATACCGC ACAGATGCGT AAGGAGAAAA TACCGCATCA
GGCGGCCTTA AGGGCCTCGT GATACGCCTA TTTTTATAGG TTAATGTCAT GATAATAATG GTTTCTTAGA CGTCAGGTGG
CACTTTTCGG GGAAATGTGC GCGGAACCCC TATTTGTTTA TTTTTCTAAA TACATTCAAA TATGTATCCG CTCATGAGAC
AATAACCCTG ATAAATGCTT CAATAATATT GAAAAAGGAA GAGT



CCCTCCCGTA TCGTAGTTAT CTACACGACG GGGAGTCAGG CAACTATGGA TGAACGAAAT AGACAGATCG CTGAGATAGG

TGCCTCACTG ATTAAGCATT GGTAACTGTC AGACCAAGTT TACTCATATA TACTTTAGAT TGATTTAAAA CTTCATTTTT
AATTTAAAAG GATCTAGGTG AAGATCCTTT TTGATAATCT CATGACCAAA ATCCCTTAAC GTGAGTTTTC GTTCCACTGA
GCGTCAGACC CC
GGCC TTTTGCTGGC CTTTTGCTCA CATGTTCTTT CCTGCGTTAT CCCCTGATTC TGTGGATAAC
CGTATTACCG CCTTTGAGTG AGCTGATACC GCTCGCCGCA GCCGAACGAC CGAGCGCAGC GAGTCAGTGA GCGAGGAAGC
GGAAG
Lac-Promotor
Lac-Operator
Ribosomen-Bindungsstelle
Ampicillin-Resistenz-Gen
pUC Plasmid-Replications-Origin
Klonierungsstellen
1. PstI
2. NcoI
3. BsrGI
4. EcoRI

Die Sequenz enthält vier silent mutations ([redacted]), die laut Sequenzanalyse eindeutig vorhanden sind:

(1) Soll: TAT, Ist: TA[redacted] -> Tyr; Codon usage (gesamtes Genom E. coli) ändert sich von 16,2 zu 12,2
(2) Soll: TAC, Ist: TA[redacted] -> Tyr; Codon usage (gesamtes Genom E. coli) ändert sich von 12,2 zu 16,2
(3) Soll: GCA, Ist: GC[redacted] -> Ala; Codon usage (gesamtes Genom E. coli) ändert sich von 20,1 zu 33,6
(4) Soll: ATT, Ist: AT[redacted] -> Ile; Codon usage (gesamtes Genom E. coli) ändert sich von 30,3 zu 25,1
(Frequenz pro Tausend)

REPLACEMENT SHEET

Sequenz des Konstrukts Domäne 3 von EF-Tu-GFP-His im Vektor pEGFP (Clontech) (SEQ ID NO:2)
(b2)

pEGFP-Vektor:
AGCGCCCAAT ACGCAAACCG CCTCTCCCCG CGCGTTGGCC GATTCATTAA TGCAGCTGGC ACGACAGGTT TCCCGACTGG
AAAGCGGGCA [illegible]
[illegible]T CACAC[illegible]A ACAGCTATGA CCATGATTAC GCCAAGCTTG
CATGC[illegible]C

Domäne 3 von EF-Tu:
GCTAAGCCGG GCACCATCAA GCCGCACACC AAGTTCGAAT CTGAAGTGTA CATTCTGTCC AAAGATGAAG GCGGCCGTCA
TACTCCGTTC TTCAAAGGCT ACCGTCCGCA GTTCTACTTC CGTACTACTG ACGTGACTGG TACCAT[illegible]GAA CTGCCGGAAG
GCGTAGAGAT GGTAATGCCG GGCGACAACA TCAAAATGGT TGTTACCCTG ATCCACCCGA TCGCGATGGA CGACGGTCTG
CGTTTCGCAA TCCGTGAAGG CGGCCGTACC GTTGGCGCGG GCGTTGTAGC TAAAGTTCTG GGCTGC

pEGFP-Vektor:
AAGCTTA[illegible]

GFP:
[illegible]TGAGCA AGGGCGAGGA GCTGTTCACC GGGGTGGTGC CCATCCTGGT CGAGCTGGAC GGCGACGTAA ACGGCCACAA
GTTCAGCGTG TCCGGCGAGG GCGAGGGCGA TGCCACCTAC GGCAAGCTGA CCCTGAAGTT CATCTGCACC ACCGGCAAGC
TGCCCGTGCC CTGGCCCACC CTCGTGACCA CCCTGACCTA CGGCGTGCAG TGCTTCAGCC GCTACCCCGA CCACATGAAG
CAGCACGACT TCTTCAAGTC CGCCATGCCC GAAGGCTACG TCCAGGAGCG CACCATCTTC TTCAAGGACG ACGGCAACTA
CAAGACCCGC GCCGAGGTGA AGTTCGAGGG CGACACCCTG GTGAACCGCA TCGAGCTGAA GGGCATCGAC TTCAAGGAGG
ACGGCAACAT CCTGGGGCAC AAGCTGGAGT ACAACTACAA CAGCCACAAC GTCTATATCA TGGCCGACAA GCAGAAGAAC
GGCATCAAGG TGAACTTCAA GATCCGCCAC AACATCGAGG ACGGCAGCGT GCAGCTCGCC GACCACTACC AGCAGAACAC
CCCCATCGGC GACGGCCCCG TGCTGCTGCC CGACAACCAC TACCTGAGCA CCCAGTCCGC CCTGAGCAAA GACCCCAACG
AGAAGCGCGA TCACATGGTC CTGCTGGAGT TCGTGACCGC CGCCGGGATC ACTCTCGGCA TGGACGAGC[illegible]AG

His-Tag:
CTTCATCACC ATCACCATCA CTAACTGTAC AAGTAA[illegible]

REPLACEMENT SHEET

pEGFP-Vektor:
CAACTGAGCG CCGGTCGCTA CCATTACCAA CTTGTCTGGT GTCAAAAATA ATAGGCCTAC TAGTCGGCCG TACGGGCCCT
TTCGTCTCGC GCGTTTCGGT GATGACGGTG AAAACCTCTG ACACATGCAG CTCCCGGAGA CGGTCACAGC TTGTCTGTAA
GCGGATGCCG GGAGCAGACA AGCCCGTCAG GGCGCGTCAG CGGGTGTTGG CGGGTGTCGG GGCTGGCTTA ACTATGCGGC
ATCAGAGCAG ATTGTACTGA GAGTGCACCA TATGCGGTGT GAAATACCGC ACAGATGCGT AAGGAGAAAA TACCGCATCA
GGCGGCCTTA AGGGCCTCGT GATACGCCTA TTTTTATAGG TTAATGTCAT GATAATAATG GTTTCTTAGA CGTCAGGTGG
CACTTTTCGG GGAAATGTGC GCGGAACCCC TATTTGTTTA TTTTTCTAAA TACATTCAAA TATGTATCCG CTCATGAGAC
AATAACCCTG ATAAATGCTT CAATAATATT GAAAAAGGAA GAGT
CCCTCCCGTA TCGTAGTTAT CTACACGACG GGGAGTCAGG CAACTATGGA TGAACGAAAT AGACAGATCG CTGAGATAGG
TGCCTCACTG ATTAAGCATT GGTAACTGTC AGACCAAGTT TACTCATATA TACTTTAGAT TGATTTAAAA CTTCATTTTT
AATTTAAAAG GATCTAGGTG AAGATCCTTT TTGATAATCT CATGACCAAA ATCCCTTAAC GTGAGTTTTC GTTCCACTGA
GCGTCAGACC CC

[redacted]GGCC TTTTGCTGGC CTTTTGCTCA CATGTTCTTT CCTGCGTTAT CCCCTGATTC TGTGGATAAC CGTATTACCG CCTTTGAGTG AGCTGATACC GCTCGCCGCA GCCGAACGAC CGAGCGCAGC GAGTCAGTGA GCGAGGAAGC GGAAG

[redacted] Lac-Promotor

[redacted] Lac-Operator

[redacted] Ribosomen-Bindungsstelle

[redacted] Ampicillin-Resistenz-Gen

[redacted] pUC Plasmid-Replications-Origin

[redacted]
5. [redacted] PstI
6. [redacted] NcoI
7. [redacted] BsrGI
8. [redacted] EcoRI

Die Sequenz enthält eine silent mutation ([redacted]), die laut Sequenzanalyse eindeutig vorhanden ist:

Soll: ATT, Ist: AT[redacted] -> Ile; Codon usage (gesamtes Genom E. coli) ändert sich von 30,3 zu 25,1 (Frequenz pro Tausend)

REPLACEMENT SHEET

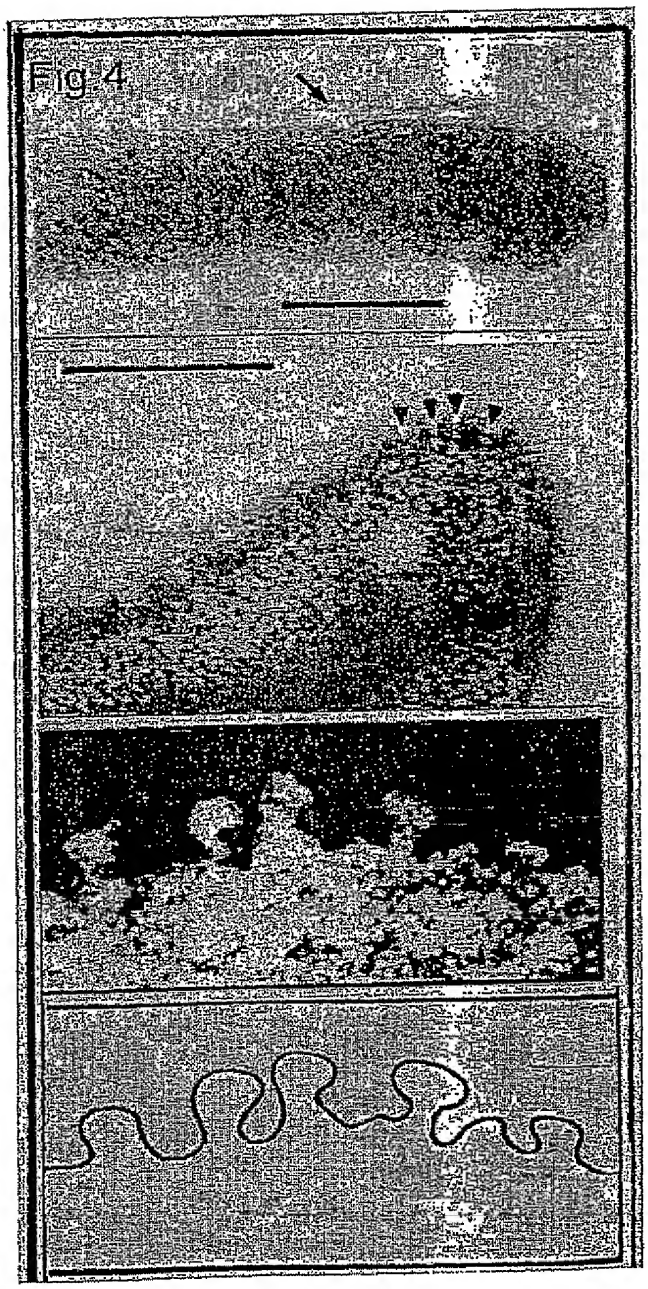
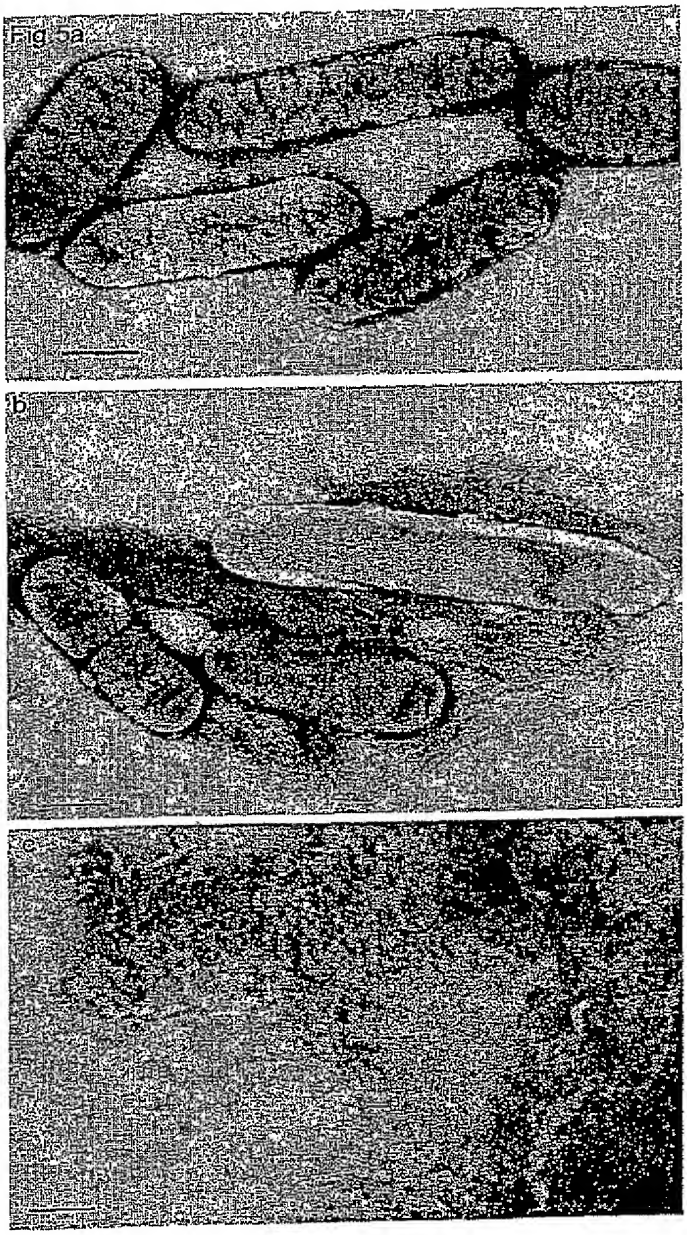


Fig 4

Fig 5a



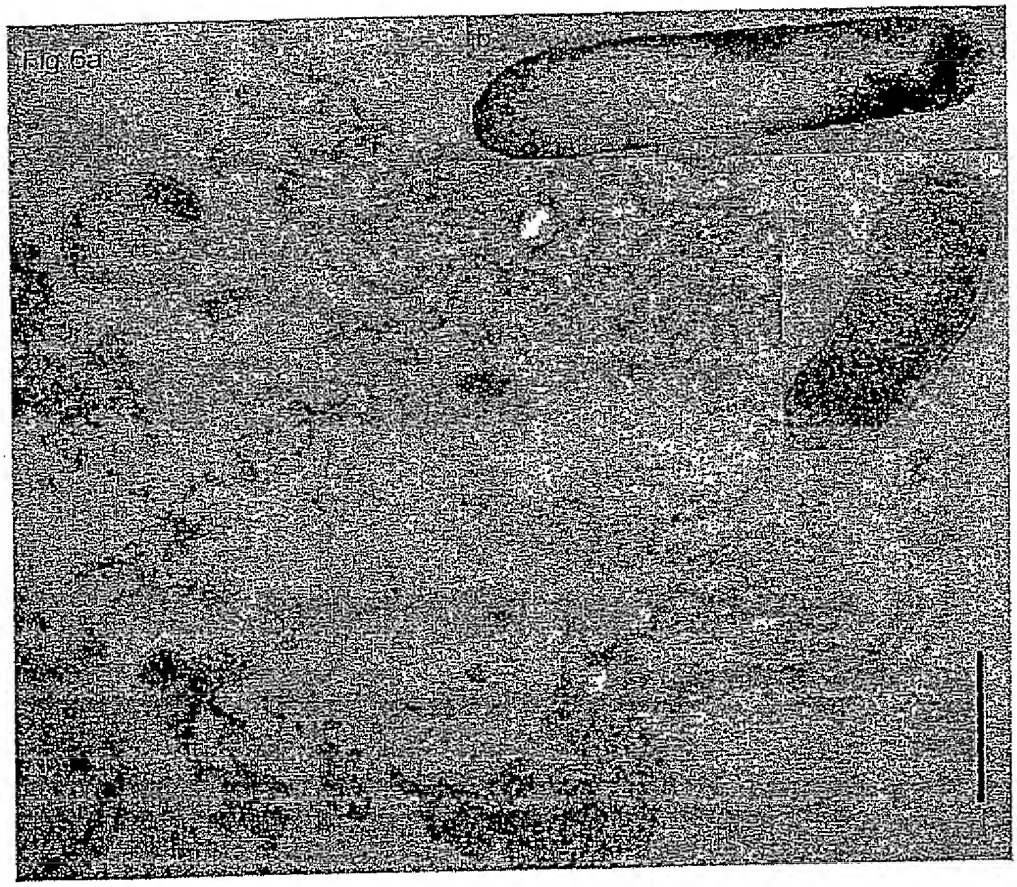
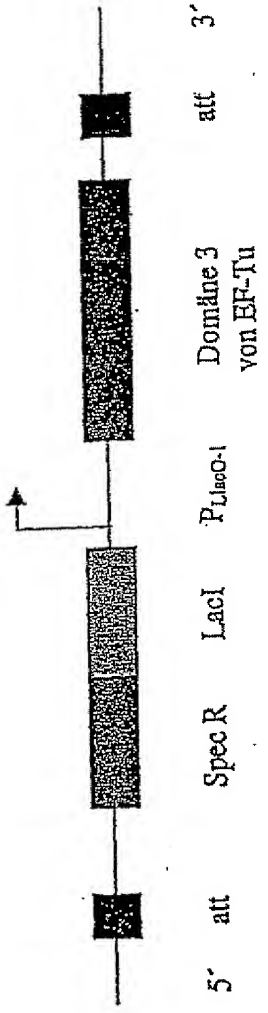


Fig. 6a



Figur 7